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Community ecology

Whole angiosperms *Wolffia columbiana* disperse by gut passage through wildfowl in South America

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For the first time to our knowledge, we demonstrate that whole angiosperm individuals can survive gut passage through birds, and that this occurs in the field. Floating plants of the genus *Wolffia* are the smallest of all flowering plants. Fresh droppings of white-faced whistling duck *Dendrocygna viduata* ($n = 49$) and coscoroba swan *Coscoroba coscoroba* ($n = 22$) were collected from Brazilian wetlands. Intact *Wolffia columbiana* were recovered from 16% of *D. viduata* and 32% of *Coscoroba* samples (total = 164 plantlets). The viability of plants was tested, and asexual reproduction was confirmed. *Wolffia columbiana* is an expanding alien in Europe. Avian endozoochory of asexual angiosperm propagules may be an important, overlooked dispersal means for aquatic plants, and may contribute to the invasive character of alien species.

1. Introduction

The dispersal of viable plant units is recognized as a vital ecosystem service provided by birds, but the great majority of the literature focuses on the dispersal of seeds by frugivorous birds [1]. It is widely assumed that only plants with a fleshy fruit are adapted for endozoochory, i.e. dispersal through the gut passage of animals [2]. However, studies of waterbirds as plant vectors bring into question this assumption, and show that endozoochory by non-frugivorous birds is important. Wildfowl (Anseriformes: ducks, geese, swans and screamers) disperse seeds of many angiosperms lacking a fleshy fruit [3], are excellent vectors for long-distance dispersal [4] and have been recently shown to disperse viable moss fragments and fern spores in their guts [5,6]. Here we demonstrate they can disperse entire angiosperms by endozoochory.

The floating, rootless plants of the genus *Wolffia* (Araceae, Lemnoideae) are the world's smallest flowering plants [7]. Like their relatives the duckweeds *Lemna*, they are widely assumed to disperse via waterbirds, but by epizoochory (i.e. attaching on the outside). Darwin [8] observed that when a duck emerges from a pond, whole *Lemna* plantlets can adhere to its feathers, and there is experimental evidence to support this [9]. Even before Darwin [8], Weddell [10] described *Wolffia brasiliensis* from plants he found on the feathers of Brazilian screamer *Anhima cornuta*. *Wolffia columbiana* has a similar native range to *W. brasiliensis* in freshwater wetlands across temperate and tropical regions from Argentina to Canada [7]. It is also alien and spreading in Europe, where it threatens native *W. arrhiza* [11].

Waterbirds can disperse plants to new habitats they cannot reach by other means [4]. The distribution of *Wolffia* in their native and introduced ranges

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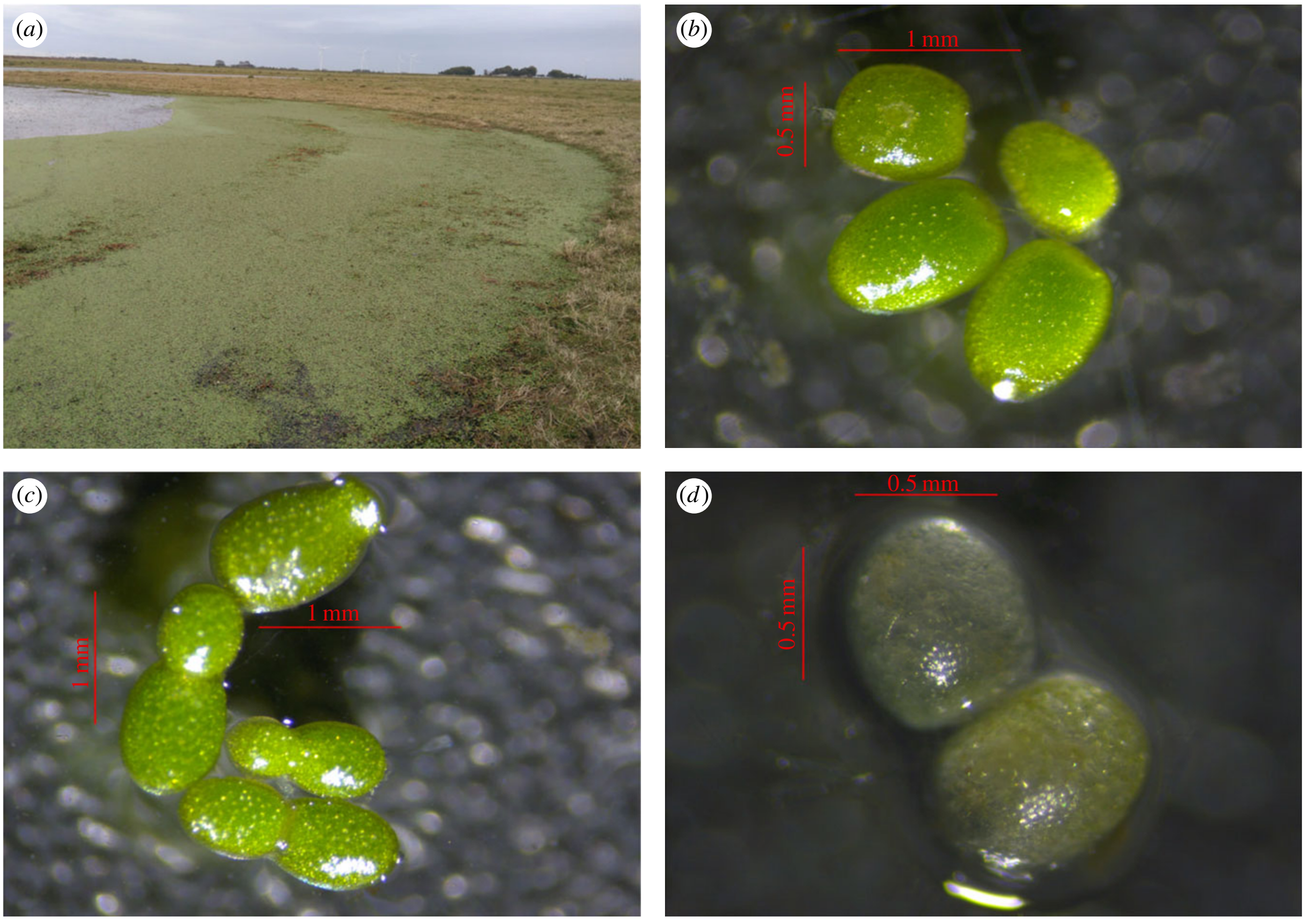

Figure 1. (*a*) Wetland where faeces of *D. viduata* with *W. columbiana* were collected. (*b*) Intact plantlets obtained from faeces, showing a healthy appearance (bright green colour and integral structure). (*c*) Seven plantlets observed after 7 experimental days, confirming asexual reproduction. (*d*) Plants that died during the experiment lost their colour.

[11,12] indicates they are effective dispersers, even though epizoochory events may be rare and constrained by desiccation of plants on plumage [13]. It has become clear that endozoochory is more frequent than epizoochory for seed dispersal by waterbirds [4,14]. Wildfowl actively feed on Lemnoideae and disperse viable *Lemna* seeds by endozoochory [4,6,15], although asexual *Lemna* somehow also disperse readily [9]. This raises the question whether endozoochory of vegetative propagules, such as *Wolffia* or *Lemna* plantlets, occurs in nature.

2. Material and methods

Fresh droppings of *Dendrocygna viduata* ($n = 49$) and *Coscoroba coscoroba* ($n = 22$) were collected between August 2017 and July 2018 (electronic supplementary material, appendix S1) in five temporary wetlands of Santa Vitória do Palmar, in southern Brazil (figure 1*a*; electronic supplementary material, appendices S2 and S3). These wetlands are situated among ricefields and cattle-grazed grasslands. They contain numerous species of emergent and floating aquatic plants [16]. *Wolffia columbiana* is common and widespread in permanent and temporary ponds, lakes and water courses. Droppings were collected from grass close to the shoreline, were not in contact with soil or water and were immediately inspected for contamination. We observed *D. viduata* and *C. coscoroba* resting close to the droppings, and given their numbers, each sample is likely to be from a different individual. *Coscoroba* droppings had a distinctive colour, size and texture different from those of any other waterbird in the area. *Dendrocygna* droppings were collected from monospecific groups.

Samples were stored in separate tubes. In the laboratory, 34 *Dendrocygna* droppings and all *Coscoroba* droppings were frozen until inspection. Fifteen droppings of *D. viduata* were kept at 4°C in the fridge until a viability experiment. All samples were carefully examined under a stereomicroscope initially, to confirm the absence of any plant propagules adhered to the exterior. Frozen faeces were then defrosted in water and examined under the stereomicroscope to separate whole plants. The 15 unfrozen droppings were processed similarly later (just before the viability experiment). Only intact *Wolffia* plants resembling live plants (i.e. with a bright green colour and integral structure, figure 1*b*) were counted and removed from the samples. Fragments were also observed in some samples, but were not quantified.

Intact *W. columbiana* plants removed from three fresh *D. viduata* droppings were counted and placed in five Petri dishes. The dropping with more plants (A) was separated into three dishes (A1, A2 and A3) to facilitate the counting of new plants produced by asexual reproduction. Plants from the other two droppings (B, C) were placed in separate dishes. All dishes were filled with filtered water from the wetland where droppings were collected. The dishes were placed in a growth chamber (12 h dark at 16°C $\pm$ 2°C, 12 h light at 26°C $\pm$ 2°C). The number of living and dead individuals was counted after 7 and 14 days. An increase in the number of plants was considered to demonstrate asexual reproduction, confirming viability.

3. Results

A total of 164 intact *W. columbiana* were observed in faecal samples (figure 1; electronic supplementary material,

Table 1. Asexual reproduction of *W. columbiana* recovered from three *D. viduata* droppings, showing changes in the cumulative number of live and dead plants after 7 and 14 days.

sample ID	initial number apparently alive	day 7				day 14			
		new plants	dead plants	live plants	total	new plants	dead plants	live plants	total
A1	5	5	—	10	10	10	1	14	15
A2	5	7	3	9	12	8	3	10	13
A3	5	—	3	2	5	3	3	5	8
B	2	2	1	3	4	5	2	5	7
C	1	—	1	—	1	—	1	—	1
total	18	14	8	24	32	26	10	34	44

appendix S1). Whole plants were observed in seven *Coscoroba* droppings (86 plants, frequency of occurrence = 31.8%, 4–24 plants per sample), and eight *D. viduata* droppings (78 plants, frequency = 16.3%, 1–31 plants per sample).

Intact *W. columbiana* were removed from three of 15 unfrozen *D. viduata* samples, and placed in Petri dishes. After 14 days, we detected vegetative reproduction in four of five dishes (with plants from two droppings). The number of living plantlets increased by 89% (figure 1 and table 1), with variation among droppings A = 93.3%, B = 150% and C = 0%.

4. Discussion

Our study provides field evidence that vegetative angiosperm propagules can be dispersed by avian endozoochory. Whole *Wolffia* plants were dispersed over an unknown distance between aquatic feeding sites and terrestrial loafing sites. Previously, asexual angiosperm propagules have only been reported from external parts of waterbirds [4,8,9]. Zoochory of asexual propagules allows dispersal outside the period of seed production and availability, e.g. facilitating the colonization of temporary wetlands after heavy rainfall.

Dendrocygna viduata is widespread in Central and South America, with an estimated population of one million [17]. Individuals fly an average of up to 4 km daily between different wetlands [18], making it an ideal plant vector. *Coscoroba coscoroba* is restricted to southern South America, with a population of 10 000–25 000 [17]. It is partially migrant, with movements of up to 1700 km [19]. Hence, endozoochory of *W. columbiana* by *D. viduata* may be a more frequent process, although *C. coscoroba* may be important for long-distance dispersal.

The high abundance and frequency of intact *W. columbiana* in faeces, and their high viability, suggest this floating plant has a high capacity to survive gut passage. Endozoochory may be more important for *W. columbiana* than epizoochory. The average retention time for wildfowl faeces is several hours [4], suggesting that wildfowl regularly disperse *Wolffia* over several kilometres during their daily movements [18]. Despite anecdotal support for epizoochory, it is unclear that floating plants both remain attached to birds and resist desiccation during extended flights. There is no risk of desiccation during endozoochory, which may provide a longer maximum retention time.

The particularly small size and simple morphology of *Wolffia* may promote endozoochory. Seeds with a smaller size and round shape are more likely to survive gut passage [4]. More research is needed to establish which angiosperm taxa can survive gut passage as whole plants or as viable fragments (as recently shown for bryophytes [5]). Experimental evidence suggests fragments of the invasive amphibious *Crassula helmsii* may disperse inside wildfowl guts [20]. Given that production of asexual vegetative propagules and an ability to grow from fragments is widespread in plants [21], dispersal of such vegetative propagules (e.g. fragments of grasses or pondweeds, or whole floating plants) by endozoochory may be an important and overlooked process. Clonality is more common in plants that establish readily outside of their native range [21], and the ability to disperse as vegetative propagules by endozoochory may increase their invasiveness. Greater resistance to desiccation has been suggested as the key to the effective dispersal of plantlets or stem fragments on the outside of animals or on boats or fishing gear [13]. Perhaps the invasive character of some species (e.g. *W. columbiana* or *Lemna minuta*) is more related to greater resistance to gut passage.

Ethics. This work was authorized by the Brazilian agency SISBIO (no. 59225-1).

Data accessibility. Datasets supporting this article have been uploaded as electronic supplementary material.

Authors' contributions. G.G.S., A.J.G., V.W., P.H. and A.L.-K. designed this study. G.G.S. and A.J.G. led the writing and the others authors contributed equally with the text. G.G.S., V.W. and P.H. collected and analysed the data. C.S. and L.M. helped in all steps and acted as coordinators. All authors read and approved the final manuscript and agree to be accountable for all the content here presented.

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